Detergent-stimulated detachment of microbial biofilms

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A microbial biofilm can be defined as a matrix-enclosed bacterial population adherent to each other and/or to surfaces or interfaces. Biofilms can develop on various natural and artificial surfaces in- and outside the human body.

On surfaces exterior to man like on teeth in the oral cavity, on contact lenses on the eye, and in certain places on the skin, typically between toe's and in armpits, almost ideal environments exist for the development of potentially pathogenic biofilms. Large numbers of people suffer the consequences of these easily emerging biofilms in the form of dental caries and periodontal diseases, corneal ulcers and fungal skin infections. An important common feature of biofilms on the exterior of the human body is their ease of accessibility. Consequently, they can be removed by a proper cleaning method.

Cleaning strategies are mostly based on a mechanical removal of biofilm components, on an antimicrobial treatment, or on a combination of both. With these measures, the amount of undesirable microbial contamination on teeth, contact lenses and skin can be minimized. However, only a limited number of people maintain the degree of motivation needed for an effective level of personal hygiene, resulting in dental caries and periodontal diseases, increasing incidence of contact lens related bacterial keratitis, and fungal skin infections. This thesis focuses on the efficacy of detergents present in personal health care products to stimulate detachment of biofilms from surfaces associated with the exterior of the human body, most notably enamel and contact lens surfaces.

The aims of this thesis, as presented in **Chapter 1.1**, are firstly to assess the efficacy of personal health care products to stimulate bacterial detachment from biomaterials surfaces using a parallel plate flow chamber system. Secondly, to evaluate the factors involved in stimulating biofilm detachment by detergent systems applied in these health care products.

In **Chapter 1.2** the use of detergents in oral health care products and their efficacy in stimulating detachment of oral biofilms are reviewed. Detergents are widely used in dentifrices and mouthrinses. They are used for their foaming capacity and for their emulsifying and cleansing properties. The most common detergent used in dentifrices is the anionic molecule sodium lauryl sulphate (SLS). Currently available mouthrinses are predominantly based on the antimicrobial effects of cationic detergents. Most studies on the efficacy of detergents as anti-plaque agents evaluate their antibacterial properties. Almost no studies have been conducted on bacterial detachment stimulated by detergents.

Chapter 1.3 reviews the application of detergents in contact lens care products. The incidence of microbial keratitis has grown rapidly, directly related to contact lens wear. A proper lens care regimen reduces the risk of developing microbial keratitis.

In **Chapter 2**, a method to quantitatively assess bacterial detachment from substratum surfaces in a parallel plate flow chamber, as stimulated by personal health care products, is presented, and applied to evaluate the effects of two mouthrinses, Hibident® and Scope®, and a pre-brushing rinse, Plax®, on the detachment of *Streptococcus sobrinus* HG 1025 adhering to enamel, with and without a salivary conditioning film. Furthermore, the influence of the hydrophobicity of the substratum on the detachment of adhering microorganisms as stimulated by the oral rinses was studied. Perfusion of the flow chamber with the two mouthrinses did not stimulate any significant detachment of adhering *S. sobrinus* HG 1025, whereas perfusion with the pre-brushing rinse stimulated up to 100% detachment. Moreover, the pre-brushing rinse Plax® was more effective in stimulating bacterial detachment from salivary conditioning films than from bare substrata. Plax®, and possibly to a lesser extent also Scope®, weakened the bond between adhering *S. sobrinus* HG 1025 and the substrata, thereby facilitating removal of adhering cells during high shear, as exerted here by the passage of a liquid-air interface through the chamber and as occurring *in vivo* by eating, speaking, drinking or swallowing. It was hypothesized that the controversies about the clinical efficacies of Plax® may be due to the inability of its, otherwise effective, detergent system to penetrate the plaque and stimulate detachment of the linking film, i.e. the initially adhering bacteria.

In **Chapter 3**, bacterial detachment studies were performed in order to examine the effects of two mouthrinses, Corsodyl® and Scope®, a pre-brushing rinse, Plax® , and its detergent components, on the detachment of a collection of linking film bacteria from saliva-coated enamel. These experiments demonstrated that bacteria adhering to saliva-coated enamel could not be stimulated to detach by perfusion of the flow chamber with two traditional mouthrinses (Corsodyl® and Scope®), whereas perfusion with a pre-brushing rinse (Plax®) or its detergent components stimulated detachment from saliva-coated enamel of a wide variety of bacterial strains. After perfusion with the pre-brushing rinse significant numbers of still-adhering bacteria could be stimulated to detach by passage of a liquid-air interface, indicating that Plax® had weakened their adhesive bond. The ability of Plax® or its detergents components to detach plaque bacteria is not always obvious from *in vivo* experiments and reports on its clinical efficacy are inconsistent. Likely, antimicrobials or detergents are unable to penetrate the plaque and reach the linking film bacteria, as demonstrated here by Fourier transform infrared spectroscopy.

In **Chapter 4**, the parallel plate flow chamber system was employed to study bacterial detachment in an ophthalmological setting. A comparison was made between the effects of two all-in-one contact lens cleaning solutions, one made for the cleaning of soft contact

lenses and the other for the cleaning of rigid lenses (RCL), and a detergent mixture, 0.2% (w/v) Tauranol and 0.25% (w/v) SLS, on the detachment of a pathogenic *Pseudomonas aeruginosa* strain from contact lenses. Furthermore, the efficacy of supplementing the RCL cleaning solution with the detergents Tauranol and SLS, was evaluated. Both all-in-one contact lens cleaning solutions stimulated minor bacterial detachment from lens surfaces with or without a tear film. The SLS/Tauranol detergent mixture, however, removed up to 95% of the adhering *P. aeruginosa* cells, while the RCL cleaning solution supplemented with detergents also stimulated significant detachment. Surface physical-chemical analysis clearly demonstrated the presence of a tear film on the contact lens surfaces, but neither remnants of the ophthalmic solutions nor of the detergents could be found.

In **Chapter 5**, the penetration of SLS through artificial dental biofilms, consisting of the cariogenic oral microorganism *Streptococcus mutans* HG 985, grown with either glucose or saccharose as the main carbon source was studied by attenuated total reflectance / Fourier transform infrared spectroscopy (ATR/FTIR). Furthermore, the detachment of initially adhering cells and growing *S. mutans* HG 985 biofilms from enamel surfaces by SLS was studied in a parallel plate flow chamber. The transport of SLS to the base of the *S. mutans* biofilms was not hindered, while moreover an accumulation of SLS near the base of the biofilms was found, suggesting that SLS was adsorbed to biofilm components. X-ray photoelectron spectroscopy confirmed the ability of *S. mutans*, grown on saccharose supplemented medium, to adsorb SLS, and simultaneously indicated that exposure of cells to SLS might lead to a loss of surface proteins. Furthermore, experiments in a parallel plate flow chamber demonstrated that initially adhering *S. mutans* HG 985 could be stimulated to detach by SLS, but that, depending on the growth stage of the biofilm, only maximally 27% of biofilm bacteria could be stimulated to detach by a 4% (w/v) SLS solution. In conclusion, *S. mutans* HG 985 biofilms detached far less upon exposure to SLS than initially adhering bacteria in the absence of growth, presumably due to adsorption of the detergent by biofilm components during penetration to the base of the biofilm.

In the **General discussion**, we have discussed ideas about the working mechanism of detergent-stimulated microbial detachment. As detergents have a great interfacial activity and may remove components, which adhere to surfaces by hydrophobic groups and by electrical forces, they may stimulate bacterial detachment by disruption of the bond between the microorganisms and the conditioning film. Adsorption of detergents by biofilm components may also occur, which could result in an accumulation of detergents in the biofilm. As described in this thesis, the recalcitrance of fully matured *S. mutans* biofilms towards

detergent-stimulated removal, as compared with the substantial detachment of initially adhering *S. mutans* cells, might parallel the growth rate dependent resistance of biofilms towards antimicrobials. In addition, we have suggested that interspecies binding is an important mechanism in the development of a complex biofilm community. It is supposed to be one of the key factors in the formation of dental plaque. On contact lens surfaces however, the population diversity is very low and interspecies binding seems of minor importance. Finally, we have proposed a research strategy for the development of personal health care products based on a detachment stimulating detergent system. After first screening a large number of possible detergents and detergent combinations in the parallel plate flow chamber, the potency of a number of selected detergents to penetrate and subsequently remove a fully developed biofilm from a substratum surface will be analyzed by ATR/FTIR. As a final step before a potential health care product, based on a detachment stimulating detergent system, could be subject to clinical trials, the efficacy of the selected detergent system could be evaluated on naturally occurring biofilms, using confocal laser scanning microscopy.

Summarizing, this thesis has illustrated that with the aid of a parallel plate flow chamber and image analyzing techniques, bacterial detachment from dental and contact lens surfaces, as stimulated by personal health care products, can be quantified under well controlled experimental conditions. It has also been described that with the aid of ATR/FTIR the efficacy of detergents to stimulate detachment of fully matured biofilms can be evaluated with respect to the penetration of detergent through the biofilm. In addition, the potency of a detergent mixture to stimulate bacterial detachment from enamel and contact lens surfaces has been demonstrated. The results support the development of personal health care products stimulating microbial detachment, as they may yield a valuable addendum to currently available personal health care products. A biofilm control strategy aimed at detachment avoids the risk of inducing microbial resistance and the possibility of eradication of an entire microbial community by antimicrobials, which might create an environment more susceptible to pathogens.